Technical Information

Middlebrook 7H9 Agar Base

Product Code: DM 1197

Application: Middlebrook 7H9 Agar Base is recommended for isolation, cultivation and sensitivity testing of *Mycobacterium tuberculosis*.

Composition**

Ingredients	Gms / Litre
Ammonium sulphate	0.500
Sodium glutamate	0.500
Sodium citrate	0.100
Pyridoxine	0.001
Biotin	0.0005
Disodium phosphate	2.500
Monopotassium phosphate	1.000
Ferric ammonium citrate	0.040
Magnesium sulphate	0.050
Calcium chloride	0.0005
Zinc sulphate	0.001
Copper sulphate	0.001
Malachite green	0.001
Agar	15.000
Final pH (at 25°C)	6.6±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Solid media for Mycobacterial cultivation may be egg-based (Lowenstein Jensen Media) or agar-based (Middlebrook Media) [1]. Dubos and Middlebrook [2] formulated new media containing oleic acid and albumin, which protect Mycobacterium from toxic agents, helping for the growth of tubercle bacilli. Middlebrook 7H9 Agar Base developed by Middlebrook and Cohn [3] is used for cultivation of Mycobacteria. This medium can also be used for sensitivity testing of Mycobacteria and for subculturing of stock cultures when OADC Growth Supplement (MS2018) and glycerol are added to Middlebrook media.

Middlebrook media consists of many inorganic salts, which help, in growth of Mycobacteria. Citric acid formed from sodium citrate helps in retaining inorganic cations in solution. Glycerol supplies carbon and energy. Middlebrook OADC Growth Supplement (MS2018) contains oleic acid, bovine albumin, sodium chloride, dextrose and catalase. Oleic acid and other long chain fatty acids are essential for metabolism of Mycobacteria. Some free fatty acids are toxic to Mycobacteria but albumin binds to those fatty acids and prevents toxic action on Mycobacteria. Dextrose serves as an energy source. Catalase neutralizes toxic peroxides. Malachite green partially inhibits other bacteria [1, 4].

Care should be taken while decontamination of the specimen. Also proper specimen collection (sputum and not saliva) should be ensured. Samples should be carefully handled to avoid contamination.

Methodology

Suspend 9.85 grams of powder media in 450 ml distilled water. 1 ml glycerol may be added if desired. Shake well & heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50-55°C and aseptically add 50 ml sterile Middlebrook OADC Growth Supplement (MS2018). Mix well and distribute as desired.

Quality Control

Physical Appearance
Light yellow to light green homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light amber coloured clear to slightly opalescent gel with greenish tinge forms in Petri plates

Reaction
Reaction of 1.97% w/v aqueous solutions at 25°C. pH : 6.6±0.2

pH range: 6.40-6.80

Cultural Response/Characteristics
DM1197: Cultural characteristics observed with added Middlebrook OADC Growth Supplement (MS2018) after an incubation at 35-37°C for 2-4 weeks.

Organism	Growth
Mycobacterium tuberculosis H37RV (25618)	Good-luxuriant
Mycobacterium fortuitum ATCC 6841	Good-luxuriant
Mycobacterium smegmatis ATCC 14468	Good-luxuriant

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2. Dubos R. J. and Middlebrook G., 1947, Am. Rev. Tuberc., 56:334.
3. Middlebrook G. and Cohn M. L., 1958, Am. J. Public Health, 48:844.
4. Finegold S. M., and Baron E. J., 1990, Bailey and Scotts Diagnostic Microbiology, 8th Ed., The C.V. Mosby Co., St. Louis.

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